S/N 09/458,862

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Allison Hubel	Examiner: Elli Peselev
Serial No.:	09/458,862	Group Art Unit: 1623
Filed:	December 10, 1999	Docket: 600.451US1
Title:	COMPOSITIONS AND METHODS FOR CRYOPRESERVATION OF PERIPHERAL BLOOD LYMPHOCYTES	

APPELLANT'S BRIEF ON APPEAL

Box AF
Commissioner for Patents
Washington, D.C. 20231

Sir:

This Brief is presented in support of the Appeal mailed March 24, 2003 from the rejection of claims 1-8, 11-12, 14, 16-17, 19-22, 24, 26-28, 30-44, and 47-58 of the above-identified application.

This Brief is being submitted in triplicate, as set forth in 37 C.F.R. § 1.192(a). Our check in the amount of $160.00 is enclosed to cover the fee for filing this Brief. Also enclosed is our check in the amount of $725.00 to cover the fee for a four month extension of time in which to file the Brief. The Commissioner is hereby authorized to charge any additional fee, or credit overpayment, to Deposit Account No. 19-0743.

Respectfully submitted,

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Date September 24, 2003 By _____
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CERTIFICATE UNDER 37 CFR § 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelop addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on this 24th day of September 2003

Name: Dawn M. Poole Signature: _____

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1. REAL PARTY IN INTEREST

The real party in interest of the above-identified patent application is the assignee, Regents of the University of Minnesota.

2. RELATED APPEALS AND INTERFERENCES

Appellant, her legal representatives, and the assignee are not aware of any other appeals or interferences which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

3. STATUS OF THE CLAIMS

For purposes of the appeal, claims 1-8, 11-12, 14, 16-17, 19-22, 24, 26-28, 30-34, 37-44, and 47-60 are pending, and claims 1-8, 11-12, 14, 16-17, 19-22, 24, 26-28, 30-34, 37-44, and 47-58 stand rejected, and are the subject of this appeal (see Appendix I).

4. STATUS OF AMENDMENTS

A Final Office Action was issued on May 30, 2002. Claims 1 and 26 were amended and claims 53-58 were added in the Request for Continued Examination dated November 27, 2002. Claims 1-2, 6, 14, 20, 26, 30, 31, 37, and 53-58 are amended, claims 59-60 are added, and claims 35-36 are canceled in the Amendment which accompanies this Brief. Claims 1-8, 11-12, 14, 16-17, 19-22, 24, 26, 28, 30-44, and 47-52 have been twice rejected.

5. SUMMARY OF THE INVENTION

Cryopreservation of cells that have been expanded and manipulated *ex vivo* is important for the clinical application of cell-based therapies (page 1, lines 28-29 of Appellant's specification). Cryopreservation facilitates pooling of cells to reach a therapeutic dose, and facilitates safety testing of both the cell product and any agents, such as recombinant viral vectors or liposomal delivery vehicles, used to genetically modify the cells (page 1, line 29-page 2, line 2). Moreover, as the genetic modification and/or expansion of cells for therapeutic use may require days to weeks for completion of the *ex vivo* culture protocol, cryopreservation facilitates the coordination of cellular therapy with donor care (page 2, lines 2-5).

Prior to the present invention, one method commonly used for the cryopreservation of therapeutically useful cells included resuspending peripheral blood lymphocytes (PBLs) in a cryopreservation medium containing 10% dimethylsulfoxide (DMSO), autologous plasma and Hank's balanced salt solution, and cooling the mixture at 1°C/minute (page 2, lines 6-10). However, DMSO cryopreservation involves the risk of DMSO-associated toxicity, and since most lymphocyte therapies require the infusion of multiple doses of cells on a regular basis, the toxic effects of DMSO can be cumulative (page 2, lines 19-20 and page 3, lines 1-3). Further, the development of appropriate solutions is not the only issue in the development of cryopreservation protocols for lymphocytes which are used therapeutically, as *in vitro* culture also influences the freezing response of cells (page 3, lines 4-7).

The instant claims are generally directed to compositions (claims 1-8, 11-12, 14, 16-17, 19-22, 24, 31-34, 37-48, 53-56, and 59) and methods (claims 26-28, 30, 49-52, 57-58, and 60) which employ the compositions, useful for the cryopreservation of freshly isolated lymphocytes, hematopoietic stem cells or lymphocytes which have been activated or genetically modified *ex vivo*. The compositions generally include an amount of arabinogalactan, or a biological or functional equivalent thereof, and optionally glycerol, which results in a high post-thaw survival rate for these cell types. The compositions do not include dimethylsulfoxide or serum.

For example, claim 37 is directed to a cryopreservation medium comprising a balanced electrolyte solution selected from the group consisting of lactated Ringer's solution, PlasmaLyte-A™, Normosol-R™, Veen-D™, Polysal®, and Hank's balanced salt solution, and at least one cryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, in an amount of 1% w/v to 40% w/v, and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified *ex vivo*, or a combination thereof, wherein the arabinogalactan, biological or functional equivalent thereof, in the medium results in a high post-thaw survival rate for the cells, and wherein the medium does not comprise dimethylsulfoxide or serum. Claim 54 is directed to a cryopreservation medium comprising a balanced electrolyte solution incorporating arabinogalactan, which is present in an amount of 1% w/v to 40% w/v, glycerol in amount of 0.5% to about 20%, and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified *ex vivo*, or a combination thereof, wherein the arabinogalactan and glycerol in the medium result in a high

post-thaw survival rate for the cells, and wherein the medium does not comprise dimethylsulfoxide or serum.

In another example, the methods of the invention include claim 26 which is directed to a method which comprises freezing a cell suspension comprising cells contacted with a cryopreservation medium comprising a balanced electrolyte solution and at least one cryoprotective agent that is arabinogalactan, a biological or functional equivalent thereof, in an amount of 1% w/v to 40% w/v, wherein the cells are freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified *ex vivo*, or a combination thereof, wherein the arabinogalactan, biological or functional equivalent thereof, in the medium results in a high post-thaw survival rate for the cells, and wherein the medium does not comprise dimethylsulfoxide or serum. Claim 57 is directed to a method which comprises freezing a cell suspension comprising cells and a cryopreservation medium comprising a balanced electrolyte solution, arabinogalactan in an amount of 1% w/v to 40% w/v, and glycerol in amount of 0.5% to about 20%, wherein the cells are freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan and glycerol in the medium result in a high post-thaw survival rate for the cells.

6. ISSUES PRESENTED FOR REVIEW

A. Whether the recited phrase "a biological or functional equivalent thereof" renders claims 1-8, 11-12, 14, 16-17, 19-22, 24, 26-28, 30-44, and 47-58 indefinite under 35 U.S.C. § 112, second paragraph.

B. Whether the recited phrase "lymphocytes modified *ex vivo*" renders claims 1-8, 11-12, 14, 16-17, 19-22, 24, 26-28, 30-44, and 47-58 indefinite under 35 U.S.C. § 112, second paragraph.

C. Whether the recited phrase "a biological or functional equivalent thereof" renders claims 1-8, 11-12, 14, 16-17, 19-22, 24, 26-28, 30-44, and 47-52 unpatentable under 35 U.S.C. § 112, first paragraph, for containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

D. Whether claims 1-8, 11-12, 14, 16-17, 19-22, 24, 26-28, 30-44, and 47-58 are anticipated under 35 U.S.C. § 102(a) over Oliver et al. (published PCT application WO 97/35472).

E. Whether claims 1-8, 11-12, 14, 16-17, 19-22, 24, 26-28, 30-44, and 47-58 are obvious under 35 U.S.C. § 103(a) over WO 97/35472.

7. GROUPING OF CLAIMS

For the rejections under 35 U.S.C. § 112, second paragraph, claims 1-8, 11-12, 14, 16-17, 19-22, 24, 26-28, 30-44, and 47-58 stand and fall together for the purposes of this appeal.

For the rejection under 35 U.S.C. § 112, first paragraph, claims 1-8, 11-12, 14, 16-17, 19-22, 24, 26-28, 30-44, and 47-52 stand and fall together for the purposes of this appeal.

For the rejections under 35 U.S.C. § 102(a) and 35 U.S.C. § 103(a), the claims are grouped as follows for the purpose of this appeal:

Group I: Claims 1-8, 11-12, 14, 16-17, 19-22, 24, 31, 33-36, 53, and 55

Group II: Claims 26-28, 30, 32, 49-52, and 58

Group III: Claims 37-48

Group IV: Claims 54 and 56

Group V: Claim 57

Group VI: Claims 59-60

Separate remarks are presented below to address the patentability of the claims in Groups I-VI.

8. ARGUMENT

I) THE CLAIMS ARE IN COMPLIANCE WITH THE REQUIREMENTS OF 35 U.S.C. § 112, SECOND PARAGRAPH

a) The Applicable Law

In rejecting a claim under the second paragraph of 35 U.S.C. § 112, it is incumbent on the Examiner to establish that one of ordinary skill in the pertinent art, when reading the claims in light of the supporting specification, would not have been able to ascertain with a reasonable degree of precision and particularity the particular area set out and circumscribed by the claims. *Ex parte Wu*, 10 U.S.P.Q.2d 2031, 2033 (B.P.A.I. 1989); *In re Moore*, 439 F.2d 1232, 169

U.S.P.Q. 236 (C.C.P.A. 1971); *In re Hammack*, 427 F.2d 1378, 166 U.S.P.Q. 204 (C.C.P.A. 1970)). The M.P.E.P. adopts this line of reasoning:

> The essential inquiry pertaining to this requirement is whether the claims set out and circumscribe a particular subject matter with a reasonable degree of clarity and particularity. Definiteness of claim language must be analyzed, not in a vacuum, but in light of:
>
> (1) The content of the particular application disclosure;
> (2) The teachings of the prior art; and
> (3) The claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made. M.P.E.P. § 2173.02.

Moreover, there is nothing intrinsically wrong in using functional language, defining something by what it does rather than by what it is, in drafting patent claims; courts have recognized the practical necessity for the use of functional language. *In re Swinehart*, 439 F.2d 210, 169 U.S.P.Q. 226, 228 (C.C.P.A. 1971).

b) Discussion of the Rejections

1. The Examiner's Position

Claims 1-8, 11-12, 14, 16-17, 19-22, 24, 26-28, 30-44, and 47-58 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. In particular, the Examiner asserts that it is not clear what is encompassed by the terminology "a biological or functional equivalent thereof" and it is not clear from the specification how lymphocytes are modified *ex vivo*. The 35 U.S.C. § 112, second paragraph, rejection over the phrase "a biological or functional equivalent thereof" is inapplicable in claims 53-58, as that phrase does not appear in those claims.

2. Appellant's Response

The specification discloses that "arabinogalactan, a biological or a functional equivalent thereof" includes naturally occurring or synthetic arabinogalactan, portions of arabinogalactan, such as degradation products, and chemically or biochemically modified arabinogalactan or portions thereof which have been modified, which are effective in a somatic cell cryopreservation medium to protect somatic cell viability properties upon freezing and thawing

of somatic cells in the medium (page 4, lines 9-16). Arabinogalactan is a natural polysaccharide, and sources of arabinogalactan are commercially available as described in PCT/US97/04764 (WO 97/35472) and U.S. Patent No. 5,116,969 (both of record). Chemically or biochemically modified arabinogalactan is disclosed in U.S. Patent Nos. 5,478,576 (of record) and 5,116,969, which were incorporated by reference into the specification.

Additionally, the claims recite that the amount of arabinogalactan, a biological or a functional equivalent thereof in the cryopreservation medium has certain specific properties. For instance, the arabinogalactan, biological or a functional equivalent thereof, is present in an amount which results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are activated or genetically modified *ex vivo*. The specification discloses that a "high survival rate" means that at least about 40%, preferably at least about 50%, more preferably at least about 60%, and even more preferably at least about 80%, of cryopreserved cells are viable upon thawing (page 4, lines 16-19). Methods to determine the viability and recovery of cells after freezing and thawing are discussed at pages 24-25 and in Examples 1 and 2 of the specification. Thus, one skilled in the art in possession of Appellant's specification can readily identify a biological or functional equivalent of arabinogalactan and determine if the biological or functional equivalent of arabinogalactan has the recited property. Hence, the phrase "biological or functional equivalent thereof" in the claims is definite.

With respect to the phrase "lymphocytes modified *ex vivo*", it is respectfully submitted that the Amendment which accompanies this Brief on Appeal, in which the claims are amended to recite "lymphocytes activated or genetically modified *ex vivo*", renders the rejection of the claims under 35 U.S.C. § 112, second paragraph, over the phrase "lymphocytes modified *ex vivo*," moot.

Appellant urges that the pending claims meet the requirements of 35 U.S.C. § 112, second paragraph. Accordingly, Appellant respectfully requests that the rejection of claims 1-8, 11-12, 14, 16-17, 19-22, 24, 26-28, 30-44, and 47-58 under 35 U.S.C. § 112, second paragraph, be reversed.

II) THE CLAIMS AND SPECIFICATION ARE IN COMPLIANCE WITH THE REQUIREMENTS OF 35 U.S.C. § 112, FIRST PARAGRAPH, AND THE EXAMINER HAS PREVIOUSLY ACQUIESCED THAT THE CLAIMS AND SPECIFICATION SATISFY THOSE REQUIREMENTS

a) The Applicable Law

The first paragraph of 35 U.S.C. § 112 states:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

To be enabling, the specification of a patent must teach those skilled in the art how to make and use the full scope of the claimed invention without undue experimentation. *Genentech Inc. v. Novo Nordisk A/S*, 108 F.3d 1361, 42 U.S.P.Q.2d 1001, 1004 (Fed. Cir. 1997). The scope of the claims must bear a reasonable correlation to the scope of enablement provided by the specification to persons of ordinary skill in the art. *Id.* Whether making or using the invention would have required undue experimentation, and thus whether the disclosure is enabling, is a matter of degree. *PPG Industries Inc. v. Guardian Industries Corp.*, 75 F.3d 1558, 37 U.S.P.Q.2d 1618, 1623 (Fed. Cir. 1996),), *reh'g denied*, 1998 U.S. App. LEXIS 30846 (Fed. Cir. 1998). The fact that some experimentation is necessary does not preclude enablement; what is required is that the amount of experimentation must not be unduly extensive. *Id.*

b) Discussion of the Rejection

1. The Examiner's Position

The Examiner rejected claims 1-8, 11-12, 14, 16-17, 19-22, 24, 26-28, 30-44, and 47-52 under 35 U.S.C. § 112, first paragraph, asserting that the specification does not reasonably provide enablement for a biological or functional equivalent of arabinogalactan.

2. Appellant's Response

As discussed above, the specification discloses that arabinogalactan, a biological or functional equivalent thereof includes naturally occurring or synthetic arabinogalactan, portions of arabinogalactan, and chemically or biochemically modified arabinogalactan or portions

thereof, which are present in an amount in a cryopreservation medium that is effective to protect somatic cell viability upon freezing and thawing (page 4, lines 10-19). Exemplary cryopreservation media are described at pages 14-16 and in Example 1 of the specification, and methods to determine the viability and recovery of cells after freezing and thawing are discussed at pages 24-25 and in Examples 1-2 of the specification. Thus, one of ordinary skill in the art in possession of Appellant's specification would be apprised of how to identify and use a biological or functional equivalent of arabinogalactan in the compositions and methods of the invention. Therefore, Appellant's specification is enabling.

Moreover, the Board is respectfully requested to consider that the same rejection was raised against the claims in the above-identified application in the Office Action dated October 25, 2000. In response to the arguments above, which were presented in the Amendment dated February 26, 2001, the rejection was withdrawn.

Hence, the Board is urged to reverse the rejection of claims 1-8, 11-12, 14, 16-17, 19-22, 24, 26-28, 30-44, and 47-52 under 35 U.S.C. § 112, first paragraph.

III) THE CLAIMS ARE NOVEL AND INVENTIVE OVER THE CITED ART

a) The Applicable Laws

35 U.S.C. § 102(a) states:

> A person shall be entitled to a patent unless
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent.

A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. *Verdegaal Bros., Inc. v. Union Oil Co. of California*, 814 F.2d 628, 2 U.S.P.Q.2d 1051, 1053 (Fed. Cir. 1987). Moreover, a reference must disclose every element of the challenged claim and enable one skilled in the art to make the anticipating subject matter. *PPG Industries, Inc. v. Guardian Industries Corp.*, 75 F.3d 1558, 37 U.S.P.Q.2d 1618, 1624 (Fed. Cir. 1996), *reh'g denied*, 1998 U.S. App. LEXIS 30846 (Fed. Cir. 1998).

35 U.S.C. § 103(a) states:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The Examiner has the burden under 35 U.S.C. § 103 to establish a *prima facie* case of obviousness. *In re Fine*, 837 F.2d 1071, 5 U.S.P.Q.2d 1596, 1598 (Fed. Cir. 1988). The M.P.E.P. contains explicit direction to the Examiner that agrees with the court's holding in *In re Fine*:

> In order for the Examiner to establish a *prima facie* case of obviousness, three base criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. M.P.E.P. § 2142 (citing *In re Vaeck*, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991)).

The Examiner must recognize and consider not only the similarities but also the critical differences between the claimed invention and the prior art. *In re Bond*, 910 F.2d 831, 15 U.S.P.Q.2d 1566, 1568 (Fed. Cir. 1990), *reh'g denied*, 1990 U.S. App. LEXIS 19971 (Fed. Cir. 1990).

Moreover, a *prima facie* case of obviousness can be rebutted by evidence of unexpected results. M.P.E.P. § 2144.09, citing *In re Papesch*, 315 F.2d 381, 137 U.S.P.Q. 43 (C.C.P.A. 1963).

b) Discussion of the Rejections

1. The Examiner's Position

The Examiner rejected claims 1-8, 11-12, 14, 16-17, 19-22, 24, 26-28, 30-44, and 47-58 under 35 U.S.C. § 102(a) as anticipated by published PCT application WO 97/35472, or

alternatively under 35 U.S.C. § 103(a) as obvious over WO 97/35472. In particular, the December 23, 2002 Office Action states that WO 97/35472 discloses that arabinogalactan is not toxic to cells and is highly compatible with a wide variety of different cell types (page 9, lines 20-24), including blood cells (page 10, line 4). It also states that WO 97/35472 discloses that cells may be frozen in arabinogalactan alone and teaches the use of DMSO as optional (page 9, lines 12-15).

The Office Action dated December 23, 2002 further states that WO 97/35472 encompasses medium containing 50% arabinogalactan, 20% arabinogalactan, 15% arabinogalactan and 10% arabinogalactan, and that medium containing 50% arabinogalactan can be used to replace serum but that a lower percentage of arabinogalactan was not successful. The Office Action dated December 23, 2002 continues stating that the instant claims encompass 40% arabinogalactan and since 40% is closer to 50% than to 20%, a person having ordinary skill in the art at the time the instant invention was made would have expected that compositions containing 40% arabinogalactan would produce results which are more similar to those produced by a composition containing 50% arabinogalactan. Finally, the Office Action dated December 23, 2002 states that the Declarations of Dr. Hubel and Dr. Bischof were not found to be persuasive since WO 97/35472 discloses cryopreservation of a wide variety of cell types, including blood cells, and since rejection over the prior art does not require that the prior art provide 100% predictability.

2. Appellant's Response

WO 97/35472 discloses that the concentration of arabinogalactan in cryopreservation media is typically from between 5 and 70% w/v, preferably between 14 and 20% w/v, although it may also be between 20 and 50% w/v, e.g., preferably between 44 and 50% w/v (page 7, lines 4-10). Optional cryopreservation agents in the media are disclosed as including DMSO, serum, and glycerol (page 7, line 25). WO 97/35472 does not disclose concentrations of glycerol useful in arabinogalactan-containing cryopreservation media.

Although WO 97/35472 indicates that the described media may be employed with a variety of cell types including human cells (page 5, line 2) and "blood" or "immune" cells (page 10, line 4 and page 11, line 30), as well as genetically altered cells (page 10, line 9), the only data provided in the WO 97/35472 specification is for seven lines of immortalized mammalian cells

(page 13, lines 1-10). These mammalian cells include three rodent epithelial cell lines, a mink fibroblast line, a human fibroblast line, a bovine endothelial cell line (CPAE cells), and a murine pre-neoplastic mammary cell line (page 13, lines 2-9).

No hematopoietic cells are represented in the seven lines of cells disclosed in WO 97/35472. Moreover, blood is known to include erythrocytes and leukocytes, and that the major classes of leukocytes are lymphocytes, monocytes, neutrophils, eosinophils, and basophils (pages 233 and 1030 of Churchill's Medical Dictionary, Churchill Livingstone, Inc. (1989), copies of which are included herewith). Further, immune cells include T cells, B cells, plasma cells and macrophage (page 920 in Churchill's Medical Dictionary, Churchill Livingstone, Inc. (1989), a copy of which is included herewith).

The seven lines disclosed in WO 97/35472 were frozen in 6 different media (Table 1). For media containing arabinogalactan, it is disclosed that arabinogalactan was prepared as a 50% w/v concentrated stock dissolved in a buffered isotonic salt solution (page 12, lines 25-27). The only base culture media disclosed in WO 97/35472 is Dulbecco's Minimal Essential Medium (page 7, lines 9-10 and page 12, line 4). It is disclosed that the arabinogalactan stock was used directly (medium 3, i.e., 50% arabinogalactan) or in combination with other components. Medium 4 has 20% arabinogalactan and 10% DMSO; medium 6 has 15% arabinogalactan and 20% serum, medium 2 has 10% arabinogalactan and 20% DMSO; and medium 5 has 10% arabinogalactan, 10% DMSO and 20% serum. Medium 1 has 10% DMSO and 20% serum (no arabinogalactan).

With respect to immediate post-thaw viability for all cell types tested, it is disclosed that there was no difference in post-thaw viability for 4 of the media relative to "the industry standard" (cell culture medium + serum + DMSO) (page 14, lines 10-13), however, cells frozen in media with arabinogalactan and serum are disclosed as having reduced viability (page 14, lines 13-15). It is also noted that there was "substantially no difference" in plating efficiency at day 1 for 6/7 of the cell types, and at 6 days post-thaw, it is disclosed that there was "substantially no difference" between treatment groups (page 14, lines 23-24, and page 14, line 29-page 15, line 2).

Table 2 shows the ranking of the media with respect to growth rates (day 6/day 1) for CPAE cells (media 3 > media 5 > media 2 > media 1 > media 4 > media 6). WO 97/35472

concludes that arabinogalactan "can be used to replace serum in a standard freezing medium, in a formulation with DMSO, for all cell types studied" and that freezing in 50% w/v arabinogalactan was better or equivalent to the standard media for 5/7 cell types tested (emphasis added; page 15, lines 20-31). Thus, although a medium with a very high percent of arabinogalactan (50%) alone was at least equivalent to standard freezing media for 5/7 cell types tested, a lower percent of arabinogalactan was not able to successfully replace DMSO in a standard freezing medium.

WO 97/35472 generally discloses that cells in cell cryopreservation media may be cooled or frozen during storage to about or below 4°C, for example to about -200°C (page 4, lines 23-25). An exemplary freezing procedure is described as resuspending cells in an arabinogalactan-containing freezing medium (1×10^6-1×10^7 cells/vial), aliquoting the resuspended cells into 1.8 ml cryovials, equilibrating the cells for about 30 minutes at 4°C, step-cooling the cells for 18 hours at -80°C, and immediately transferring the cooled cells to liquid nitrogen (-196°C) (page 8, lines 19-28 and Example 2).

The Board is requested to consider that methods and compositions useful to cryopreserve one cell type are not necessarily useful to preserve other cell types, as each cell type has different biological and physical properties. For example, in the Rule 132 Declaration executed by Dr. Allison Hubel, the inventor of the claimed invention and an eminent researcher in the area of cryopreservation of cells for therapeutic purposes, which was filed with an Amendment on August 27, 2001 (Appendix II), Dr. Hubel states that a variety of interrelated factors influence the ability of cells to survive the stresses of freezing and thawing including (1) the composition of the cryopreservation solution; (2) the temperature history of the sample during cooling (e.g., cooling rate); and (3) the biological and biophysical characteristics of the cell/tissue being frozen (paragraph 5 of the Hubel Declaration). Dr. Hubel notes that some cell types, such as granulocytes (a "blood" cell), cannot be cryopreserved at all (paragraph 5 of the Hubel Declaration). Note that neutrophils, eosinophils and basophils are included as granulocytes (page 810 in Churchill's Medical Dictionary, Churchill Livingstone, Inc. (1989), a copy of which is included herewith).

Dr. Hubel also states that during rapid cooling, there is insufficient time for water to leave the cell in response to the increase in extracellular solution concentration resulting from the removal of water experienced during freezing (paragraph 7 of the Hubel Declaration).

Undercooling of the cell relative to the extracellular solution results in intracellular ice formation, a lethal event, and slow cooling can result in excessive dehydration of the cell that is also damaging to the cell (paragraph 7 of the Hubel Declaration). Dr. Hubel also states that the relative water content of a cell during freezing is a function of the cell type (with each cell type exhibiting its own unique biophysical characteristics) and the function of the solution composition in which the cell is suspended (paragraph 7 of the Hubel Declaration). Evidence that survival and cooling rate vary with the composition, and that different cell types have different cooling rates when present in the same freezing medium, is provided in paragraphs 8 and 9 of the Hubel Declaration. Dr. Hubel concluded that a general disclosure of a method of freezing cells which employs an arabinogalactan-containing freezing medium useful for one cell type does not enable a method or composition useful for another cell type due to differences in the biophysical and biological properties of each particular type of cell (paragraph 11 of the Hubel Declaration)

In this regard, the Board is also requested to consider page 127 of Sputtek et al. (In: Clinical Applications in Cryobiology, CRC Press, 1991) (of record), where it is noted that the conditions employed to freeze red blood cells were suboptimal for the preservation of viable white blood cells. Further, in Hubel (Transfusion Med. Rev., 11, 224 (1997)) (of record), it is disclosed that the membrane permeability parameters for a number of blood cell types including lymphocytes, were found to be distinctive (see Table 1 of the Hubel article). In addition, Figure 3 in the Hubel article provides data showing that freshly isolated $CD34^+$ cells and cultured, transduced $CD34^+$ cells have different physical characteristics at different temperatures. These included water permeability, cell volume and the osmotically inactive cell volume fraction.

Yet further evidence that different cell types exhibit different behaviors in any particular cryopreservation medium is shown in Table 3 and 4 of Appellant's specification. Tables 3 and 4 show the differences in cell recovery for activated peripheral blood lymphocytes versus cultured peripheral blood lymphocytes, and genetically altered peripheral blood lymphocytes versus normal peripheral blood lymphocytes, in the same arabinogalactan-containing cryopreservation medium and relative to DMSO-containing medium.

In addition, the Board is respectfully requested to consider the Rule 132 Declaration executed by Dr. John C. Bischof, an eminent researcher in the area of cryobiology, which was

filed with a Response on October 30, 2002 (Appendix II). In the Declaration, Dr. Bischof states that, prior to the filing of the above-identified application, there was still a considerable need for the development and refinement of cryopreservation solutions and protocols (paragraph 3 of the Bischof Declaration). Dr. Bischof explains that, in particular, solutions and protocols developed for cells which were amenable to cryopreservation and employable in cellular-based therapies, frequently resulted in suboptimal levels of post-thaw viability and, more often than not, employed protective agents that were harmful upon infusion (paragraph 3 of the Bischof Declaration).

Moreover, Dr. Bischof states that the fundamental physical phenomena present during freezing are strongly influenced by the composition of the freezing solution and the cell type being preserved and that protocols and solutions developed for one cell type may not be appropriate for another (paragraph 4 of the Bischof Declaration). Based on the data in WO 97/35472, Dr. Bischof concludes that one skilled in the relevant field would not have a reasonable expectation that the protocols and solutions disclosed in WO 97/35472 would be useful for other cell types and, in particular, for cells such as freshly isolated lymphocytes, hematopoietic stem cells, or *ex vivo* modified lymphocytes (paragraphs 4 and 6 of the Bischof Declaration).

Further, Dr. Bischof states that most clinical and commercial applications of cryopreserved cells or tissues require a threshold level of post-thaw viability, e.g., 50% post thaw viability (paragraph 5 of the Bischof Declaration). Dr. Bischof concludes that WO 97/35472 provides no reasonable expectation that the use of any particular arabinogalactan-containing solution would result in a threshold level of post-thaw viability for cells employed in cellular-based therapies (paragraph 6 of the Bischof Declaration).

Thus, because the concentration of cryoprotective agent useful in a cryopreservation medium is based on the biophysical properties of each cell type, and so varies with cell type, WO 97/35272 fails to teach or suggest an arabinogalactan-containing cryopreservation composition that is effective to cryopreserve freshly isolated lymphocytes, hematopoietic stem cells or lymphocytes which are activated or genetically modified *ex vivo*, and which results in a high post-thaw survival rate for those cells or methods which employ such a composition (claims 1-8, 11-12, 14, 16-17, 19-22, 24, 26-28, 30-34, 37-44, and 47-60 (Groups I-VI). In particular, WO

97/35472 does not teach or suggest a composition comprising an amount of arabinogalactan, or a biological or functional equivalent thereof, and freshly isolated lymphocytes, hematopoietic stem cells or lymphocytes which are activated or genetically modified *ex vivo*, which results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells or lymphocytes which are activated or genetically modified *ex vivo* (claims 1-8, 11-12, 14, 16-17, 19-22, 24, 31, 33-36, 53, and 55; Group I), or a cryopreservation method which employs such a composition and results in a high post-thaw survival rate for freshly isolated lymphocytes, hematopoietic stem cells or lymphocytes which are activated or genetically modified *ex vivo* (claims 26-28, 30, 32, 49-52, and 58; Group II).

Nor does WO 97/35472 disclose or suggest a cryopreservation medium comprising a balanced electrolyte solution, at least one cryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, in an amount of 1% w/v to 40% w/v, and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the balanced electrolyte solution is lactated Ringer's solution, PlasmaLyte-A™, Normosol-R™, Veen-D™, Polysal®, or Hank's balanced salt solution (claims 37-48; Group III), electrolyte solutions which closely approximate the composition of extracellular mammalian physiological fluids (page 14, lines 18-23 of Appellant's specification).

Further, WO 97/35472 clearly fails to teach or suggest a cryopreservation medium comprising 1% w/v to 40% w/v of arabinogalactan, and 0.5% to about 20% glycerol, which results in a high post-thaw survival rate for freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are activated or genetically modified *ex vivo* (claims 54 and 56; Group IV) or a cryopreservation method which employs such a composition (claim 57; Group V). In addition, WO 97/35472 does not disclose or suggest an arabinogalagactan-containing cryopreservation composition that is effective to cryopreserve freshly isolated lymphocytes, hematopoietic stem cells or lymphocytes which are activated or genetically modified *ex vivo*, and which results in an about 40% post-thaw survival rate for those cells, or a method of using such a composition (claims 59-60; Group VI).

Thus, the claims recite elements which are not expressly or inherently set forth in WO 97/35472, and so cannot be anticipated thereby.

Moreover, to establish the required reasonable expectation of success to support a *prima facie* case of obviousness, at least some degree of predictability is required. M.P.E.P. 2143.02. The Examiner has provided no evidence that the general disclosure in WO 97/35472 would provide one of ordinary skill in the art with a reasonable expectation that arabinogalactan-containing compositions are useful to cryopreserve all cell types, much less freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are activated or genetically modified *ex vivo*. In contrast, Appellant has provided extensive evidence that one of ordinary skill in the art at the time of Appellant's filing would not reasonably expect that a particular cryopreservation media useful for one cell type would be useful for another cell type (see Sputtek et al., the Hubel article, and the Declarations of Dr. Hubel and Dr. Bischof, discussed above).

Further, even if one of ordinary skill in the art had believed it was possible that arabinogalactan, a biological or functional equivalent thereof, could cryopreserve freshly isolated lymphocytes, hematopoietic stem cells or lymphocytes which are activated or genetically altered *ex vivo*, there would be no reason to expect that cryopreservation of freshly isolated lymphocytes, hematopoietic stem cells or lymphocytes which are activated or genetically altered *ex vivo* in a medium containing certain amounts of arabinogalactan, a biological or functional equivalent thereof, result in about 40% post-thaw survival rate for those cells (claims 59-60; Group VI).

Therefore, Appellant urges that the present invention is not anticipated by or obvious in view of WO 97/35472, and so requests that the 35 U.S.C. § 102(a) and § 103(a) rejections be reversed.

9. SUMMARY

It is respectfully submitted that the pending claims are definite. Moreover, it is respectfully submitted that the pending claims are fully enabled. Further, it is respectfully submitted that the cited art does not anticipate the claimed invention or render the claimed

invention obvious. Accordingly, reversal of the Examiner's rejections and allowance of all of the claims is respectfully requested.

Respectfully submitted,

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CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Mail Stop AF, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on this 24th day of September, 2003.

Dawn M. Poole
Name

_____
Signature

APPENDIX I: The Claims on Appeal

1. A cryopreservation medium comprising a balanced electrolyte solution incorporating at least one cryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, which agent is present in an amount of 1% w/v to 40% w/v, and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan, biological or functional equivalent thereof, in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are activated or genetically modified *ex vivo*.

2. The cryopreservation medium of claim 1, 53 or 54 wherein the cells are peripheral blood lymphocytes or lymphocytes which are activated or genetically modified *ex vivo*.

3. The cryopreservation medium of claim 1 that comprises arabinogalactan.

4. The cryopreservation medium of claim 1 further comprising a cryoprotective agent that penetrates the cell membrane.

5. The cryopreservation medium of claim 4 wherein the cryoprotective agent that penetrates the cell membrane is glycerol or propylene glycol.

6. The cryopreservation medium of claim 1 further comprising a cryoprotective agent other than the arabinogalactan, biological or functional equivalent thereof, which does not penetrate the cell membrane.

7. The cryopreservation medium of claim 1 which does not comprise protein.

8. The cryopreservation medium of claim 1 which is infusible.

11. The cryopreservation medium of claim 1 wherein the cells are human cells.

12. The cryopreservation medium of claim 1 wherein the cells are non-human vertebrate cells.

14. A composition suitable for administration to a human, comprising a suspension of cells in a cryopreservation medium comprising a balanced electrolyte solution incorporating at least one cryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, and a cryoprotective agent that penetrates the cell membrane, wherein the arabinogalactan, or a biological or functional equivalent thereof, is present in an amount of 1% w/v to 40% w/v, wherein the cells are freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan, biological or functional equivalent thereof, results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are activated or genetically modified *ex vivo*.

16. The composition of claim 14 wherein the cells are peripheral blood lymphocytes.

17. The composition of claim 14 wherein at least one of the cryoprotective agents is arabinogalactan.

19. The composition of claim 14 wherein the cryoprotective agent that penetrates the cell membrane is glycerol or propylene glycol.

20. The composition of claim 14 further comprising a cryoprotective agent other than arabinogalactan, biological or functional equivalent thereof, which does not penetrate the cell membrane.

21. The composition of claim 14 which does not comprise protein.

22. The composition of claim 14 which is infusible.

24. The composition of claim 14 wherein the cells are human cells.

26. A method for preserving cells comprising:

(a) contacting cells with a cryopreservation medium comprising a balanced electrolyte solution and at least one cryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, in an amount of 1% w/v to 40% w/v, to yield a cell suspension, wherein the cells are freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan, biological or functional equivalent thereof, in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are activated or genetically modified *ex vivo*; and

(b) freezing the cell suspension to yield a frozen cell suspension.

27. The method of claim 26 further comprising thawing the frozen cell suspension under conditions that maintain cell viability.

28. The method of claim 26 wherein the cells are human cells.

30. The method of claim 26, 57 or 58 wherein the cells are peripheral blood lymphocytes or lymphocytes which are activated or genetically modified *ex vivo*.

31. A frozen composition comprising i) a balanced electrolyte solution, ii) at least one cryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, in an amount of 1% w/v to 40% w/v, and iii) freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified *ex*

vivo, or a combination thereof, wherein the composition does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan, biological or functional equivalent thereof, results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are activated or genetically modified *ex vivo*.

32. A frozen hematopoietic cell-containing composition made according to the method of claim 26.

33. The cryopreservation medium of claim 5 wherein the cryoprotective agent that penetrates the cell membrane is glycerol.

34. The cryopreservation medium of claim 33 wherein the concentration of glycerol is about 1% to about 3%.

37. A cryopreservation medium comprising a balanced electrolyte solution, at least one cryoprotective agent that is arabinogalactan, or a biological or functional equivalent thereof, in an amount of 1% w/v to 40% w/v and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, wherein the balanced electrolyte solution is selected from the group consisting of lactated Ringer's solution, PlasmaLyte-A™, Normosol-R™, Veen-D™, Polysal®, and Hank's balanced salt solution, and wherein the arabinogalactan, biological or functional equivalent thereof, results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are activated or genetically modified *ex vivo*.

38. The cryopreservation medium of claim 37 wherein the lymphocytes are peripheral blood lymphocytes.

39. The cryopreservation medium of claim 37 wherein the agent is arabinogalactan.

40. The cryopreservation medium of claim 37 further comprising a cryoprotective agent that penetrates the cell membrane.

41. The cryopreservation medium of claim 40 wherein the cryoprotective agent that penetrates the cell membrane is glycerol or propylene glycol.

42. The cryopreservation medium of claim 37 further comprising a cryoprotective agent other than arabinogalactan or a biological or functional equivalent thereof which does not penetrate the cell membrane.

43. The cryopreservation medium of claim 37 which does not comprise protein.

44. The cryopreservation medium of claim 37 which is infusible.

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47. The cryopreservation medium of claim 37 wherein the cells are human cells.

48. The cryopreservation medium of claim 37 wherein the cells are non-human vertebrate cells.

49. The method of claim 26 wherein the medium comprises arabinogalactan.

50. The method of claim 26 further comprising a cryoprotective agent that penetrates the cell membrane.

51. The method of claim 50 wherein the cryoprotective agent that penetrates the cell membrane is glycerol or propylene glycol.

52. The method of claim 26 wherein the lymphocytes which are modified *ex vivo* are activated lymphocytes or genetically modified lymphocytes.

53. A cryopreservation medium comprising a balanced electrolyte solution incorporating at least one cryoprotective agent that is arabinogalactan, which is present in an amount of 1% w/v to 40% w/v, and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan in the medium results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are activated or genetically modified *ex vivo*.

54. A cryopreservation medium comprising a balanced electrolyte solution incorporating arabinogalactan, which is present in an amount of 1% w/v to 40% w/v, glycerol in amount of 0.5% to about 20%, and freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan and glycerol in the medium result in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are activated or genetically modified *ex vivo*.

55. A frozen composition comprising i) a balanced electrolyte solution, ii) at least one cryoprotective agent that is arabinogalactan in an amount of 1% w/v to 40% w/v, and iii) freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified *ex vivo*, or a combination thereof, wherein the composition does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan in the composition results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are activated or genetically modified *ex vivo*.

56. A frozen composition comprising i) a balanced electrolyte solution, ii) at least one cryoprotective agent that is arabinogalactan in an amount of 1% w/v to 40% w/v, iii) glycerol in amount of 0.5% to about 20%, and iv) freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified *ex vivo*, or a combination thereof, wherein the composition does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan and glycerol in the composition result in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are activated or genetically modified *ex vivo*.

57. A method for preserving cells comprising: freezing a cell suspension comprising cells and a cryopreservation medium comprising a balanced electrolyte solution, arabinogalactan in an amount of 1% w/v to 40% w/v, and glycerol in amount of 0.5% to about 20%, to yield a cell suspension, wherein the cells are freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan and glycerol in the medium result in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are activated or genetically modified *ex vivo*.

58. A method for preserving cells comprising:

(a) contacting cells with a cryopreservation medium comprising a balanced electrolyte solution and at least one cryoprotective agent that is arabinogalactan, in an amount of 1% w/v to 40% w/v, to yield a cell suspension, wherein the cells are freshly isolated lymphocytes, hematopoietic stem cells, lymphocytes which are activated or genetically modified *ex vivo*, or a combination thereof, wherein the medium does not comprise dimethylsulfoxide or serum, and wherein the arabinogalactan in the composition results in a high post-thaw survival rate for the freshly isolated lymphocytes, hematopoietic stem cells, or lymphocytes which are activated or genetically modified *ex vivo*; and

(b) freezing the cell suspension at a cooling rate of about 1° to about 10° C/minute to yield a frozen cell suspension.

59. The medium of claim 1, 37, 53 or 54 wherein the post-thaw survival rate is at least 40%.

60. The method of claim 26, 57 or 58 wherein the post-thaw survival rate is at least 40%.

APPENDIX II: Office Actions, Amendments, Responses and Rule 132 Declarations

1. Office Action dated October 25, 2000
2. Amendment dated February 26, 2001
3. Final Office Action dated April 16, 2001
4. Amendment dated August 27, 2001 and Rule 132 Declaration of Dr. Allison Hubel
5. Advisory Action dated September 7, 2001
6. Request for Continued Examination dated October 16, 2001
7. Office Action dated February 22, 2002
8. Amendment dated May 2, 2002
9. Final Office Action dated May 30, 2002
10. Response dated October 30, 2002 and Rule 132 Declaration of Dr. John Bischof
11. Advisory Action dated November 18, 2002
12. Request for Continued Examination dated November 27, 2002
13. Office Action dated December 23, 2002